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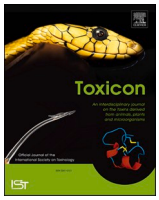

Localization modalities for botulinum neurotoxin injection

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ABSTRACT

Botulinum toxin is a targeted therapeutic that acts primarily at the site of injection. Various approaches have been taken to guide injection into the selected muscle, gland, organ or other body area. Guidance methodologies that can be used in the office setting for skeletal muscle and salivary gland percutaneous injection include uninstrumented manual needle placement, electromyography (EMG), electromyography with electrical stimulation (e-stim), ultrasound (US) and combined guidance (US + EMG or US + e-stim). This article reviews the advantages, disadvantages, and accuracy of each method and the impact of each guidance technique on therapeutic outcome for muscle and salivary gland injections. Overall, manual placement may suffice for large and superficial muscles, however, all instrumented techniques improve accuracy. Electromyography can uniquely provide information on muscle activity, while e-stim can aid injection in patients who cannot voluntarily activate a selected muscle. Ultrasound is the only technique that can visualize internal structures, allowing identification of a safe trajectory for injection of small or deep targets that might otherwise be inaccessible.

1. Introduction

Botulinum toxin (BoNT) is a focal therapeutic that must be injected directly into the affected body area, e.g. muscles (skeletal muscle, sphincters, or bladder), glands (sweat, salivary, or lacrimal glands), skin or subcutaneous tissues, or lesions. Currently approved BoNT formulations for clinical use are provided in solution or freeze-dried or vacuum-dried forms that require reconstitution with a diluent before injection. BoNTs exert their therapeutic effects through internalization into cholinergic presynaptic neurons where they disrupt neurotransmitter release. While BoNTs act predominantly at the local level, central nervous system effects may also contribute to their clinical effectiveness. Significant systemic effects are uncommon. Of the eight clostridial BoNT serotypes, only types A and B have received U.S. FDA approval for clinical use. Despite differences in formulation, dose and intracellular site of action, all clinical A and B toxins necessitate site-specific injection and have comparable clinical effectiveness.

Optimizing therapeutic outcome with BoNT hinges on careful selection of the target for injection, BoNT formulation, dose, and dilution, and injection technique. BoNT treatment should be provided in the context of collaboration with the patient to manage expectations and to establish realistic goals.

In the management of dystonia and spasticity, BoNT is administered intramuscularly. Muscle selection is the first step and is crucial to successful outcome. Evaluation for muscle selection includes a comprehensive history and physical examination focused on muscle tone, voluntary and involuntary movement and function (Karp and Alter, 2017).Electromyography may also be helpful by detecting inappropriate activity in muscles that should be at rest or, conversely, a lack of activity in a muscle that otherwise appears to be contributory to the dystonia (Alter et al., 2020). For hypersalivation, toxin is injected into salivary glands. Evaluation focuses on the pattern of hypersalivation at rest (constitutive salivation) compared to that elicited by external stimuli (e.g. food or smells). Thus, specific muscles or glands are chosen for injection based on individualized examination and outcome goals.

Once the sites for injection are selected, accurate delivery of the toxin is desirable. If toxin is injected close to, but not directly into, the selected site, its therapeutic effect must rely on diffusion to reach the intended target. Utilizing diffusion to reach the site of action at the neuromuscular or neuroglandular junction, may necessitate a higher toxin dose and

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cause more off-target adverse effects, such as weakness in unintended muscles.

This paper discusses the techniques currently available to guide BoNT injection, reviews the data on the accuracy of needle placement and impact of guidance techniques on BoNT effectiveness, and examines the possible advantages and drawbacks associated with each technique. We focus on BoNT injection guidance methodologies that can be used in the office setting for skeletal muscle and salivary gland percutaneous injection: uninstrumented manual needle placement, electromyography (EMG), electromyography with electrical stimulation (e-stim), and ultrasound (US) and a combination of guidance methods (EMG + US, E-stim + US) (Table 1). Localization techniques are employed to ensure accurate delivery of toxin where intended, maximizing its effect while minimizing adverse and off-target effects. They can also help prevent damage to internal structures from the needle during the procedure. Techniques for cystoscopic, endoscopic or intra-operative injections and those using fluoroscopy, CT or MRI imaging are beyond the scope of this discussion.

2. Background

Electromyography was used for the first clinical application of BoNT for strabismus, pioneered by Alan Scott. The 1980 report "Botulinum toxin injection into extraocular muscles as an alternative to strabismus surgery" (Scott, 1980) states:

> "From the tip of the EMG needle we record the muscle activity to determine if the injection is going into the muscle. The needle is inserted into the extraocular muscle region, the eye then turns into the field of action of that muscle to activate the motor units, and then the needle is advanced until it is in the area of the neuromuscular junction (about 2.5 cm posterior to the insertion) and the EMG response indicates it to be within the muscle itself. After dozens of electromyographic needle insertions, we find extraocular muscles still elusive, and believe it would be difficult to inject reliably without electromyographic guidance."

EMG was similarly employed in the early BoNT treatment procedure for dystonia including spastic dysphonia, oromandibular dystonia (Brin et al., 1987), craniocervical dystonia (Jankovic and Orman, 1987), and hand dystonia (Lees et al., 1992), although Brin et al. noted that they discontinued the use of EMG for oromandibular and lingual dystonia once they gained experience in those injections (Brin et al., 1987). Schiano et al. published the first report on the use of ultrasound to guide injection, using endoscopic US for BoNT treatment of esophageal achalasia (Schiano et al., 1996).

Table 1
Guidance techniques for botulinum toxin injection.

Manual needle placement using anatomic landmarks[a]
Electromyography
Without electrical stimulation[a]
With electrical stimulation (E-stim)[a]
Imaging
Ultrasound[a]
Fluoroscopy
CT scanning
MRI
Combined guidance
Ultrasound + EMG[a]
Ultrasound + E-stim[a]
Direct observation
Visual for skin or surface lesions
Endoscopy

[a] Office-based procedures discussed in this article.

2.1. Manual localization

Manual localization utilizes the clinician's knowledge of standard anatomy, visual inspection and palpation to identify anatomic landmarks referenced to muscles and other structures under the skin. This method requires no specialized equipment and can be used to inject some non-muscle targets, such as salivary glands, as well as muscles. Most clinicians learn basic human anatomy during schooling that can be supplemented by continuing education courses and training with injection models or simulators. Standard anatomy textbooks are an important resource. Diagnostic EMG guidebooks are helpful, while anatomic atlases written explicitly for BoNT injection provide more specific illustrations and instructions on where to insert the needle in relation to surface landmarks for the structures to be injected (Jost, 2019; Alter and Wilson, 2014). However, manual localization is limited in its ability to account for normal individual anatomic variations and, importantly, changes due to diseases or conditions that can distort anatomy such as spasticity, atrophy, and contractures. Similarly, restrictions in patient positioning due to contractures or involuntary movements limit reliance on standard anatomic reference guides (Alter et al., 2015). Assessing the depth of non-surface targets when relying solely on surface anatomic landmarks can be particularly challenging.

2.2. Electromyography (EMG)

Electromyography came into wide clinical use for evaluation of neuromuscular conditions in the 1950s. For EMG, an insulated needle electrode is inserted through the skin into a muscle to detect neuromuscular activity that can be conveyed to the examiner as an auditory signal and/or visual waveforms. For EMG-guided injections, an insulated injecting monopolar electrode needle is used for dual purposes: obtaining muscle signals and delivering the neurotoxin. Monopolar EMG-injection needles for neurotoxin injection are commercially available with an integrated lead wire and insulation along the needle shaft so that signal is captured only at the needle tip. For injection, reference and ground electrodes are placed on the skin. While a standard EMG machine can be used, handheld EMG devices may offer more convenience. The precise needle location is ascertained by instructing the patient to voluntarily contract the target muscle, generating a muscle interference pattern on EMG.

BoNT acts on the presynaptic neuron at the neuromuscular junction and therefore may be more effective when placed at motor endplates. Injecting at the motor endplate might 1) decrease outcome variability by having a more consistent injection location at each injection session, 2) decrease toxin dose requirement and, subsequently, cost, and 3) decrease the risk of off-target effects. Shaari et al. found that injection at the motor endplate maximized paralysis, quantified by glycogen staining of the rat tibialis anterior muscle following nerve stimulation (Shaari

and Sanders, 1993). Injection 0.5 cm from the endplate led to 50 % less paralysis. Similarly, Lapatki et al. found that BoNT efficacy, measured by a decrease in compound muscle action potential amplitude following injection into the foot extensor digitorum brevis muscle in healthy adults, decreased with the distance from the motor endplate (Lapatki et al., 2011). A 1 cm increase in distance from the endplate diminished the effect of toxin by 46 %.

EMG is the only localization method that can identify motor endplates. In clinical practice, however, motor endplates are rarely sought. Endplates are difficult and/or time consuming to find, requiring multiple needlesticks and adding time to the procedure. Injection at the endplate is often painful, adding to patient discomfort. Rather, injections are directed more generally to muscle innervation zones, which have been identified for many muscles relevant to neurotoxin treatment (Van Campenhout and Molenaers, 2011; Childers, 2004; Delnooz et al., 2014). Gracies et al. found that biceps spasticity was reduced more with injections along the endplate band or high volume injection (Gracies et al., 2009). Delnooz et al. reported that half-dose endplate band-targeted injection into sternocleidomastoid and splenius capitis lead similar improvement as full dose non-endplate zone EMG guided injection (Delnooz et al., 2014). However, Im et al. compared injections at the innervation band to more distal injections in gastrocnemius in adults with spasticity due to stroke (Im et al., 2014). Injections were successful in both cohorts with no differences in post-treatment muscle hyperactivity or clinical benefit. Mayer et al. compared motor point injection to multisite injection into biceps and brachioradialis for upper limb spasticity and found no difference between approaches in clinical outcomes (Mayer et al., 2008).

As well as assisting with accurate localization, EMG may help inform which muscles to inject in patients with spasticity or dystonia. If EMG detects ongoing activity indicating over-contraction in a muscle that should be at rest, that muscle may require injection. Conversely, if a muscle is silent when dystonic posturing is present, injection of that muscle is likely not warranted.

In addition to knowledge of functional anatomy required for manual localization, EMG requires equipment and training. EMG can increase patient discomfort if multiple needle sticks or repositioning is needed and may increase procedure time. Important limitations of EMG are that it cannot distinguish between adjacent muscles if they perform the same action, as in the layered muscles of the posterior neck that contribute to neck extension and rotation, or in the presence of co-contraction. In patients with upper motor neuron syndromes, the usefulness of EMG alone for muscle localization is often limited by mass synergies, co-contraction in non-targeted muscles, and by some patients' inability to voluntarily isolate and contract the target muscle. EMG guidance is also only applicable to muscle targets.

2.3. EMG with electrical stimulation (e-stim)

An electrophysiologic localization method that does not depend on the ability to selectively voluntarily activate a muscle is e-stim. Using an EMG machine or handheld device with stimulation capability, a pulse of gradually increasing intensity can be transmitted through the EMG needle until a visible twitch or action is generated in the target muscle. E-stim may be especially useful in patients who cannot voluntarily contract the target muscle or in the presence of co-contraction. It can also aid identification of individual fascicles of finger flexor and extensor muscles. Similar to EMG without stimulation, e-stim may require repeated needle repositioning and increased procedure time. Beyond the discomfort of EMG and the injection, the stimulation pulses can be painful; sedation is often required to perform e-stim in children. Targeting errors can occur when using e-stim when the needle is in an untargeted muscle but twitch is observed in the target muscle due to volume conduction from excessive stimulation intensity. Combined e-stim-ultrasound guidance is also utilized by some clinicians whereby the clinician observes for visible muscle contraction on the ultrasound display screen while applying low levels of stimulation via the insulated EMG needle. This provides added confirmation that the needle is within the target muscle (Alter et al., 2015).

2.4. Ultrasound (US)

Ultrasound employs high frequency soundwaves to generate images of internal anatomic structures based on their echogenic properties. US facilitates visualization of internal organ position, depth, shape, and size. During injection, US enables real-time visual tracking of needle advancement and can show the flow of injectate into the target. Among instrumented office techniques, US uniquely allows visualization of non-muscle targets as well as displaying neurovasculature and other structures to avoid, enabling delineation of safe trajectories to deep targets. It can also aid identification of muscle fibrosis, atrophy, displacement and anatomic variation. The adoption of US in the clinic has been limited by the cost of US machines and associated materials (e.g. gel, US transducers, and transducer covers) and the specialized training required. A limitation of US is that it does not provide information on muscle activity. However, it can be safely and effectively combined with EMG to integrate anatomic imaging with assessment of muscle activity.

3. Comparative accuracy and effectiveness of guided injections

To aid clinician choice of a guidance method that optimizes patient outcome, data on the accuracy of each technique and the impact of accuracy on toxin effectiveness are essential. We identified 43 papers published between 1992 and 2023 that investigated the accuracy and/or efficacy of manual localization, EMG, e-stim and/or ultrasound for toxin injections and 4 review articles. Of these, 21 studies assessed injection accuracy, while 22 evaluated efficacy in adult or pediatric populations across various conditions and injection targets (Table 2).

3.1. Accuracy of needle localization

Twenty-one studies investigated the precision of needle placement with different techniques, with 11 studies performed using cadavers (Haig et al., 2003; Hallgren et al., 2008; Boon et al., 2011; Schnitzler et al., 2012; Yun et al., 2015; Ko et al., 2020; Kim et al., 2021; Kreisler et al., 2021; Stecco et al., 2021; Vejbrink Kildal et al., 2023; So et al., 2017) and 10 observational studies in patients (Tables 3 and 4). (Speelman and Brans, 1995; Ajax et al., 1998; Molloy et al., 2002; Chin et al., 2005; Yang et al., 2009; Loens et al., 2020; Picelli et al., 2012a, 2012b; Kreisler et al., 2020; Montminy et al., 2022) This section discusses studies on needle placement into muscles. Studies on the accuracy of localization methods in the treatment of hypersalivation are discussed separately below.

3.1.1. Cadaver studies

In 7 cadaver studies of muscle targets, the accuracy of needle placement was assessed by injecting dye under manual or US guidance followed by pathological dissection to verify dye location; one study also incorporated CT scanning to detect the location of simultaneously injected iodinated contrast. One cadaveric study employed surface landmarks to guide manual placement of fine wires into lower limb muscles and then used CT imaging to confirm wire positioning. Another cadaver study examined both fine wire placement and dye injection into suboccipital neck musculature. The latter study and the remaining cadaver reports used anatomic dissection for injection site identification. All of the cadaver studies evaluated the accuracy of manual localization; seven of the studies compared manual placement to ultrasound guidance (Table 3).

The precision of manual needle placement in the cadaveric reports exhibited considerable variability, ranging from 39 to 100 %, whereas US-guided insertions had a consistently higher accuracy, achieving correct placement in 88–100 % of cases. While US guidance improves

Table 2
Publications on the accuracy and/or effectiveness of targeting methods for BoNT injection.

47 papers 1992–2023
- 21 evaluated accuracy
- 11 cadaver studies
- 10 patient observational studies
- 22 evaluated efficacy
- 20 prospective
- 1 retrospective
- 1 prospective/retrospective
- 4 systematic reviews

Population
- 35 adult
- 8 pediatric

Target
- 6 upper extremity
- 13 lower extremity
- 4 upper and lower extremity
- 12 neck
- 1 subscapularis
- 1 internal anal sphincter
- 1 facial muscles
- 5 salivary glands

Table 3
Accuracy of needle placement: Cadaver studies.

Reference	Target	N insertions	Method	Confirmation	Accuracy mean (range)		Comments
					Manual	Ultrasound	
Haig (Mayer et al., 2008)	leg	263	Fine wire insertion into lower limb muscles	Dissection	57 % (0–100 %)		17 % of injection within 5 mm or pierced undesirable structures (nerves, tendons, blood vessels, joints)
Hallgren (Haig et al., 2003)	neck	181	Fine wire insertion or dye into suboccipital neck muscles	Dissection	80.6 % (63–83 %)		
Boon (Hallgren et al., 2008)	leg	112	Fine wire insertion into 14 lower limb muscles	CT scanning	39 % (0–100 %)	96 % (semitendinosis 50 %; other muscles 100 %)	Less experienced injectors had incorrect trajectory to muscle more often than experienced injectors
Schnitzler (Boon et al., 2011)	leg	121	Dye injection gastrocnemius	Dissection	43 %		Missed injections were too deep or superficial
Yun (Schnitzler et al., 2012)	leg	32	Dye injection tibialis posterior, peroneus longus, long and short heads of biceps femoris	Dissection	71.9 % (50–93.8 %)	96.9 % (93.8–100 %)	
Ko (Yun et al., 2015)	neck	72	Dye injection	Dissection	62.5 % (54–79 %)	97 % (96–100 %)	
Kim (Ko et al., 2020)	neck	36	Dye injection into sternocleidomastoid	Dissection		100 %	
Kreisler (Kim et al., 2021)	neck	156	Dye and CT contrast injection multiple neck muscles	Dissection and CT scan	48 % (40–58.3 %)	88.2 % (83–100 %)	
Stecco (Kreisler et al., 2021)	Subsca-pularis	4	Dye injection medial and lateral approaches to subscapularis	Dissection	100 %	100 %	
Vejbrink Kildal (Stecco et al., 2021)	face; lacrimal glands	182	Dye injection into 3 facial muscles and lacrimal glands	Dissection	50 % (46–54 %) all targets 8 % lacrimal gland	88 % (62–100 %) all targets 62 % lacrimal gland	23 % placements targeted to depressor anguli oris stained the facial artery

accuracy relative to manual methods, it is not perfect. Even with US, needle misplacement can occur, most likely due to poor demarcation of muscle boundaries or failure to visualize the needle tip. Predictably, the accuracy of needle placement was better for larger and surface muscles than for smaller or deeper muscles. Regardless of the placement technique used, when misplacement occurred, the dye or wire location was predominantly either deep or superficial to the target rather than lateral, underscoring the challenge of using surface landmarks to estimate needle depth. Three of the studies compared the accuracy of experienced and inexperienced practitioners, finding little difference between them (Boon et al., 2011; Schnitzler et al., 2012; Yun et al., 2015).

Several caveats should be considered when extrapolating the cadaver data to clinical practice. Cadaveric injection can be confounded by post-mortem changes in tissues, the body cannot be positioned to aid approach to the target, voluntary activation of a muscle is not possible, and dye has different flow characteristics in the cadaver than toxin through living tissue.

3.1.2. *Observational studies*

The nine observational studies focused on manual placement, with one additionally evaluating e-stim. These studies did not compare localization modalities: one technique guided needle placement and a second technique was used to verify needle location. Observational studies conducted prior to 2009 relied on EMG or e-stim to confirm needle location, while studies published after 2009 used ultrasound for verification (Table 4).

Accuracy into individual muscles was highly variable, ranging from as low as 11 % for the tibialis posterior in children with cerebral palsy to

Table 4
Accuracy of needle placement: Observational studies.

Reference	N insertions	Population	Placement method	Assessment method	Accuracy mean % (range)
Speelman (So et al., 2017)	540	Adult cervical dystonia	manual	EMG	72 % (47–83 %)
Ajax (Speelman and Brans, 1995)	381	Adult dystonia/spasticity; upper/lower extremity	manual	EMG	71 % (56–86 %)
Molloy (Ajax et al., 1998)	38	Adult focal hand dystonia	manual	EMG	37 % (45 % if fascicles excluded)
Chin (Molloy et al., 2002)	1372	Ped cerebral palsy, upper/lower extremity	manual	e-stim	11–78 %
Yang (Chin et al., 2005)	272	Ped cerebral palsy, gastrocnemius only	manual	US	79 % (medial 93 %, lateral 65 %)
Picelli (Loens et al., 2020)	324	Adult spasticity, gastrocnemius only	manual/e-stim	US	manual = e-stim: 92 % medial gastrocnemius, 79 % lateral gastrocnemius; E-stim better for proximal lateral gastrocnemius
Picelli (Picelli et al., 2012a)	164	Adult spasticity, upper extremity	manual	US	51 % (39–63 %)
Kreisler (Picelli et al., 2012b)	264	Adult cervical dystonia	manual	US	68–100 %
Montminy (Kreisler et al., 2020)	36	Ped constipation, internal anal sphincter	manual	US	40 %

100 % for large and superficial muscles. As with cadaveric insertions, manual placement into large and superficial muscles was more accurate than that into small or deep muscles.

Many factors undermine the accuracy of manual insertions. While helpful, anatomic atlases reflect an idealized or average anatomy derived from healthy individuals. In clinical practice, patient-specific and condition-associated variations alter the anatomy. Henzel et al. mapped ultrasound-derived coordinates for 4 upper limb muscles onto the skin surface of patients with spasticity, comparing them to the injection sites described in an EMG guide. They found significant discrepancies between the EMG-recommended sites and the ultrasound-mapped locations for several forearm flexor muscles (Henzel et al., 2010).

4. Does accurate localization matter? Comparative efficacy of localization methods

Whether more accurate localization translates into better patient outcome has been a matter of debate, as many clinicians achieve acceptable results with manual injection alone (Barbano, 2001; Jankovic, 2001). The impact of EMG, e-stim and/or US guidance on BoNT efficacy and effectiveness have been explored in adult dystonia (Comella et al., 1992; Geenen et al., 1996; Hong et al., 2012; Wu et al., 2016; Kutschenko et al., 2020; Lungu et al., 2022; Tyslerowicz et al., 2022; Kreisler et al., 2022) (Table 5) and spasticity (Picelli et al., 2012a, 2014; Lungu et al., 2022; Ploumis et al., 2014; Santamato et al., 2014; Zeuner et al., 2017; Turna et al., 2020; Hauret et al., 2023) (Table 6) and in pediatric spasticity (Py et al., 2009; Xu et al., 2009; Kwon et al., 2010; Kaushik et al., 2018) (Table 7). These studies varied widely in their outcome measures, which likely contributes to the variability of results.

In adult cervical dystonia, single studies have demonstrated that EMG guidance is superior to manual placement, while US surpassed both e-stim and manual placement (Comella et al., 1992; Hong et al., 2012; Tyslerowicz et al., 2022). Wu et al. did not find a difference in therapeutic benefit when comparing manual and EMG guidance, however, they observed a higher incidence of dysphagia with manual injection (Wu et al., 2016). Hong et al. similarly reported a 34 % incidence of dysphagia associated with manual injection but no dysphagia with US guidance (Hong et al., 2012).

In focal hand dystonia, Geenen et al. found e-stim non-inferior to EMG (Geenen et al., 1996). Similarly, Lungu et al. reported no significant difference in outcome between US and e-stim for focal hand dystonia or upper extremity spasticity (Lungu et al., 2022).

For adult spasticity, US outperformed manual placement in three of four comparative efficacy studies (Picelli et al., 2012a; Ploumis et al., 2014; Santamato et al., 2014). EMG was also better than manual placement (Ploumis et al., 2014). In the comparison between e-stim and US, four out of five studies found no significant difference in outcome; one indicated that US was superior (Picelli et al., 2012a, 2014; Lungu et al., 2022; Turna et al., 2020; Hauret et al., 2023).

In pediatric spasticity, three of 4 studies comparing US against manual and/or e-stim guidance found US superior (Py et al., 2009; Xu et al., 2009; Kwon et al., 2010). One study did not find a difference in outcome comparing manual and US guidance, however only gastrocnemius was injected in that study (Kaushik et al., 2018).

Systematic reviews have aided interpretation of the literature on the comparative effectiveness of various injection guidance modalities. Grigoriu et al. conducted a review of the literature on instrument-guided injections published between 1980 and 2014, selecting 10 papers for analysis (Grigoriu et al., 2015). They found that guided techniques were superior to manual placement in the neurotoxin treatment of spasticity and dystonia, but little difference between instrumented guidance methods. Chan et al. identified 4 clinical trials published between 1990 and 2016 that compared 2 or more localization techniques for adult spasticity (Chan et al., 2017). They found Level 1 evidence that instrumented injection yield superior outcomes compared to manual placement. Asimakidou et al. reviewed the literature up until December 2022, identifying six clinical trials that used 2 or more localization techniques in adult spasticity, used the modified Ashworth scale for evaluation, and assessed outcome 2–6 weeks after injection (Asimakidou and Sidiropoulos, 2023). Using a Bayesian network meta-analysis to rank techniques, they found that all guided approaches led to better outcome than manual placement with US outperforming e-stim and EMG, with minimal difference between e-stim and EMG.

5. Hypersalivation

Intraglandular injection of BoNT is used to treat hypersalivation (Table 8). So et al. evaluated the accuracy of needle placement into adult parotid and submandibular glands using dissection for verification. Their findings indicated an accuracy of 79 % for manual placement and 96 % for US guidance into the parotid gland, while submandibular gland accuracy was 50 % for manual and 92 % for US-guided insertion (So et al., 2017). Similarly, Loens et al. reported a 74 % accuracy rate for parotid gland when the needle was placed manually and verified with US. (Loens et al., 2020) Two of three studies assessing the impact of guidance technique on treatment outcome found that US-guided injections improved hypersalivation more than manually-guided injection (Dogu et al., 2004; Svetel et al., 2009; Pires et al., 2023).

Table 5
Comparative efficacy of localization methods on BoNT outcome in adult dystonia. Shading indicates significantly better outcome.

Reference	Design	N patients	Rando-mized	Masking	Popula-tion	Condition	Primary outcome measure	Results	Localization Method				Comments
									Manual	EMG	E-tim	US	
Comella[42]	P	52	yes	double	adult	Cervical dystonia	modified TWSTRS; patient report	71% improved overall, greater benefit with EMG.	X	X			
Geenen[43]	P	12	yes	single	adult	Focal hand dystonia	MRC ≥1 grade decrease	No significant difference		X	X		single muscle injected
Hong[44]	P/R	5	no	none	adult	Cervical dystonia	Incidence of dysphagia	35% incidence dysphagia EMG; 0% US		X		X	
Wu[45]	P	65	yes	single	adult	Cervical dystonia	Tsui score	No difference at 4,8,12 weeks; EMG better at 16 weeks	X	X			more dysphagia with manual
Kutschenko[46]	R	75	n/a	n/a	adult	Cervical dystonia	Incidence of dysphagia	US did not decrease incidence of dysphagia	X			X	
Lungu[47*]	P	19	yes	single	adult	upper extremity spasticity and focal hand dystonia	Visual analog scale of change	No difference e-stim vs US; less discomfort with US			X	X	
Tyslerowicz[48]	P	35	no	single	adult	Cervical dystonia	TWSTRS, Tsui score, CDQ-24, CGI	US better than manual	X			X	
Kreisler[49]	P	123	no	not stated	adult	Cervical dystonia	CDIP-58	No difference at 1 month manual vs. US	X			X	US patients included many receiving US guidance because manual had been unsuccessful.

*Included patients with upper limb spasticity and/or dystonia; also in Table 6
Abbreviations: P: prospective, R: retrospective, n/a: not applicable, TWSTRS: Toronto Western Spasmodic Torticollis Rating Scale, MRC: Medical Research Council scale, CDIP-58: Cervical Dystonia Impact Profile

6. Effect of guidance techniques on safety

Botulinum toxin diffuses from the site of injection with the extent of spread influenced by factors such as dose, dilution and volume of injectate (Brodsky et al., 2012). In cadaver studies utilizing ink injection to assess injection accuracy, ink often spread outside of the target, especially with high volume injections.

Diffusion to off-target structures can cause adverse effects such as dysphagia due to weakness in uninjected pharyngeal muscles. Precision in targeting the injection can help limit these off-target effects. As noted above, Wu et al. observed a reduced incidence of dysphagia with BoNT injection into neck muscles for cervical dystonia when using EMG guidance compared to manual injection (Wu et al., 2016). When switching 5 patients with cervical dystonia from EMG-to US-guided injections, Hong et al. found that the incidence of dysphagia dropped from 35 % to 0 % (Hong et al., 2012). Kutschenko et al. found that US reduced the incidence of dysphagia, but did not eliminate it (Kutschenko et al., 2020). Conversely, others have found no significant difference in dysphagia across different guidance techniques (Kreisler et al., 2022).

Adverse effects with BoNT administration can arise not only from the pharmacological action of the toxin, but also from the needle puncture. EMG, e-stim and US can help assure accurate needle placement, however, only US provides real-time visualization to ensure that the needle does not traverse or enter unintended, and possible risky structures. A report of ischiorectal fossa abscess following transvaginal BoNT injection of obturator internus and pubococcygeus without instrumented guidance may represent improper needle insertion through the muscle, tracking bacteria into the ischiorectal fossa (Brueseke and Lane, 2012). EMG or US confirmation that the needle was in muscle may have prevented this complication.

The long history of BoNT injection with manual, EMG or e-stim guidance attests to its safety in most circumstances. However, inability to ensure safe access to the target has precluded injection of deep muscles with these techniques even though such muscles may contribute significantly to disability. For example, the longus coli is often involved in dystonic anteroflexion of the neck. Its deep paravertebral location behind the thyroid, trachea, esophagus, carotid artery and jugular vein makes blind injection unsafe. Ultrasound is the sole office-based

Table 6
Comparative efficacy of localization methods on BoNT outcome in adult spasticity. Shading indicates significantly better outcome.

Reference	Design	N patients	Rando-mized	Masking	Condition	Primary outcome measure	Results	Localization Method				Comments
								manual	EMG	E-stim	US	
Picelli[35]	P	47	yes	single	Lower extremity spasticity	MAS, TS, PROM ankle dorsiflexion	US >manual on MAS/PROM; US>Estim PROM; Estim = manual	X		X	X	Gastrocnemius only injection
Picelli[50]	P	60	yes	single	Upper extremity spasticity	MAS, TS, PROM	US=Estim>manual	X		X	X	
Ploumis[51]	P	27	yes	single	Upper and lower extremity spasticity	MAS, modified Barthel Index	EMG> manual	X	X			
Santamato[52]	P	30	yes	single	Upper extremity spasticity	MAS, finger position at rest	US> manual	X			X	
Zeuner[53]	P	23	yes	single	Upper extremity spasticity	MAS, Barthel Index, EQ-5D, Disability assessment scale, VAS	No difference between techniques	X	X		X	Study under-powered
Turna[54]	P	40	no	none	Lower extremity spasticity	MAS, Brunnstrom staging system, 20m walk, Barthel Index	No difference between techniques			X	X	Gastrocnemius, soleus, tibilais posterior only
Lungu[47,*]	prospective	19	yes	single	Upper extremity spasticity /focal hand dystonia	VAS change	No difference between techniques; less discomfort with US			X	X	
Hauret[55]	prospective	29	yes	single	Lower extremity spasticity	TS	No difference between techniques			X	X	

*Included patients with upper limb spasticity and/or dystonia; also in Table 5
Abbreviations: P: Prospective, MAS: Modified Ashworth Scale, TS: Tardieu Spasticity Scale, PROM: passive range of motion,EQ-5D: EuroQuality of Life=5 Dimension), VAS: visual analog scale

Table 7
Comparative efficacy of localization methods on BoNT outcome in pediatric spasticity. Shading indicates significantly better outcome.

Reference	Design	N patients	Rando-mized	Masking	Condition	Primary outcome measure	Results	Localization Method				Comments
								manual	EMG	E-stim	US	
Py[56]	P	54	no	none	CP: Lower extremity spasticity	Knee angle increase >5°	More benefit in US	X			X	More effective in age <6
Xu[57]	P	65	yes	single	CP: Lower extremity spasticity	Ankle PROM, MAS, Composite spasticity score, functional measures	More benefit on all outcomes with e-stim	X		X		All groups received concurrent physical therapy
Kwon[58]	P	23	Pseudo-randomized	single	CP: Lower extremity spasticity	MAS, MTS, Selective Motor Control, Physician's Rating Scale	Both groups improved over baseline at 1 mo; US>e-stim at 3 mo			X	X	Triceps surae only injected
Kaushik[59]	P	30	yes	none	CP: Lower extremity spasticity	MAS, Gross Motor Function Measure	No difference between techniques	X			X	Gastrocnemius only injection

Abbreviations: P: prospective, CP: cerebral palsy, MAS: Modified Ashworth Scale, MTS: Modified Tardieu Scale, PROM, passive range of motion

modality able to help the clinician delineate a safe pathway for injection of longus colli, usually through a frontal transthyroid or anterolateral approach (Tyslerowicz and Jost, 2019; Farrell et al., 2020). Similarly, the maxillary artery normally varies in its location relative to the lateral pterygoid muscle. Ultrasound can identify maxillary artery location so that an intraoral approach to the lateral pterygoid avoiding the artery can be used if needed (Unal et al., 2022).

7. Conclusions

When providing BoNT injections in the office setting, EMG, e-stim and US enhance the accuracy of needle placement, particularly in small and deep muscles. Despite the use of a guidance technique, it is still possible to miss the target especially with EMG and e-stim, where the depth of structures is more frequently misjudged than their lateral location. It is important to recognize that the different guidance methodologies provide distinct information and are not mutually exclusive; each offers unique advantages and drawbacks. Combining techniques, such as using US for anatomy visualization with EMG for assessing muscle activity as a guide to muscle selection, may optimize the accuracy and effectiveness of BoNT injection.

While BoNT can be safely and effectively injected in many patients

Table 8
Comparative accuracy and efficacy of localization methods on BoNT outcome hypersalivation. Shading indicates significantly better outcome.

Reference	Purpose	Design	N participants or insertions	Randomized	Masking	Population	Target	Outcome measure	Results	Localization Method		Verification Method for accuracy studies	
										manual	US	US	dissection
So[28]	Accuracy	Cadaver	36	N/A	yes	Adult	Parotid	N/A	Parotid accuracy 79% manual, 96% US (NS)	X	X		X
							Submandibular	N/A	Submandiular accuracy 50% manual, 92% US				
Loens[34]	Accuracy	Observational	21	no	none	Adult	Parotid	N/A	Parotid accuracy 74% manual	X		X	
Dogu[63]	Efficacy	P	15	yes	none	Adult	Parotid	Saliva production	US decreased saliva more than manual at week 1	X	X		
Svetel[64]	Efficacy	P	19	no	none	Adult	Parotid	UPDRS salivation item	No difference in techniques	X	X		
Pires[65]	Efficacy	R	16 participants; 23 procedures	N/A	N/A	Pediatric	Parotid & Submandibular	Stonel Greenberg Scale	Improvement 1 & 3 months with US; no improvement manual. US better overall and in submandibular gland. No difference in parotid gland	X	X		

Abbreviations: P: prospective, R: retrospective, N/A: not applicable, UPDRS: Unified Parkinson's Disease Rating Scale

with manual, uninstrumented guidance, published studies indicate that more accurate injection localization increases effectiveness and there is growing expert consensus advocating that an instrumented guidance technique be incorporated into clinical practice (Albanese et al., 2015; Wissel et al., 2009; Heinen et al., 2006). To maximize treatment outcomes, training programs should ensure that clinicians have access to the necessary equipment, instruction, and practice to be able to utilize these techniques.

CRediT authorship contribution statement

Barbara Illowsky Karp: Writing – review & editing, Writing – original draft, Methodology, Conceptualization. **Ann Ly:** Writing – review & editing. **Katharine E. Alter:** Writing – review & editing.

Ethical statement

This review complies with all applicable ethical regulations.
This review did not involve the participation of human subjects.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Dr. Karp is a Guest Editor for this journal. She has no competing financial interests related to this paper. Dr. Alter is a Guest Editor for this journal. She has received royalty payments from Springer, Inc for books on techniques for the injection of botulinum toxin. Dr. Ly has no competing financial interests related to this paper. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

References

Ajax, T., Ross, M.A., Rodnitzky, R.L., 1998. The role of electromyography in guiding botulinum toxin injections for focal dystonia and spasticity. J. NeuroEng. Rehabil. 12, 1–4.

Albanese, A., Abbruzzese, G., Dressler, D., et al., 2015. Practical guidance for CD management involving treatment of botulinum toxin: a consensus statement. J. Neurol. 262 (10), 2201–2213. https://doi.org/10.1007/s00415-015-7703-x.

Alter, K.E., Wilson, N.A., 2014. Botulinum Neurotoxin Injection Manual. Demos Medical, New York.

Alter, K., 2015. Guidance techniques for botulinum toxin injections: a comparison. In: Brashear, A., Elovic, E. (Eds.), Spasticity, 2 ed. Springer, New York.

Alter, K.E., Nichols, S.A., 2020. Guidance techniques for chemodenervation. In: Murphy, K.P., McMahon, M.A., Houtrow, A.J. (Eds.), Pediatric Rehabilitation. Principles and Practice, sixth ed. Springer Publishing, New York, pp. 124–145.

Asimakidou, E., Sidiropoulos, C., 2023. A bayesian network meta-analysis and systematic review of guidance techniques in botulinum toxin injections and their hierarchy in the treatment of limb spasticity. Toxins 15 (4). https://doi.org/10.3390/toxins15040256.

Barbano, R.L., 2001. Needle EMG guidance for injection of botulinum toxin. Needle EMG guidance is useful. Muscle Nerve 24 (11), 1567–1568. https://doi.org/10.1002/mus.1185.

Boon, A.J., Oney-Marlow, T.M., Murthy, N.S., Harper, C.M., McNamara, T.R., Smith, J., 2011. Accuracy of electromyography needle placement in cadavers: non-guided vs. ultrasound guided. Muscle Nerve 44 (1), 45–49. https://doi.org/10.1002/mus.22008.

Brin, M.F., Fahn, S., Moskowitz, C., et al., 1987. Localized injections of botulinum toxin for the treatment of focal dystonia and hemifacial spasm. Mov. Disord. 2 (4), 237–254. https://doi.org/10.1002/mds.870020402.

Brodsky, M.A., Swope, D.M., Grimes, D., 2012. Diffusion of botulinum toxins. Tremor Other Hyperkinet Mov (N Y) 2. https://doi.org/10.7916/D88W3C1M.

Brueseke, T.J., Lane, F.L., 2012. Ischiorectal fossa abscess after pelvic floor injection of botulinum toxin. Am. J. Obstet. Gynecol. 206 (3), e7–e8. https://doi.org/10.1016/j.ajog.2011.12.023.

Chan, A.K., Finlayson, H., Mills, P.B., 2017. Does the method of botulinum neurotoxin injection for limb spasticity affect outcomes? A systematic review. Clin. Rehabil. 31 (6), 713–721. https://doi.org/10.1177/0269215516655589.

Childers, M.K., 2004. Targeting the neuromuscular junction in skeletal muscles. Am. J. Phys. Med. Rehabil. 83 (10 Suppl. l), S38–S44. https://doi.org/10.1097/01.phm.0000141129.23219.42.

Chin, T.Y., Nattrass, G.R., Selber, P., Graham, H.K., 2005. Accuracy of intramuscular injection of botulinum toxin A in juvenile cerebral palsy: a comparison between manual needle placement and placement guided by electrical stimulation. J. Pediatr. Orthop. 25 (3), 286–291. https://doi.org/10.1097/01.bpo.0000150819.72608.86.

Comella, C.L., Buchman, A.S., Tanner, C.M., Brown-Toms, N.C., Goetz, C.G., 1992. Botulinum toxin injection for spasmodic torticollis: increased magnitude of benefit with electromyographic assistance. Neurology 42 (4), 878–882. https://doi.org/10.1212/wnl.42.4.878.

Delnooz, C.C., Veugen, L.C., Pasman, J.W., Lapatki, B.G., van Dijk, J.P., van de Warrenburg, B.P., 2014. The clinical utility of botulinum toxin injections targeted at the motor endplate zone in cervical dystonia. Eur. J. Neurol. 21 (12), 1486. https://doi.org/10.1111/ene.12517 e98.

Dogu, O., Apaydin, D., Sevim, S., Talas, D.U., Aral, M., 2004. Ultrasound-guided versus 'blind' intraparotid injections of botulinum toxin-A for the treatment of sialorrhoea in patients with Parkinson's disease. Clin. Neurol. Neurosurg. 106 (2), 93–96. https://doi.org/10.1016/j.clineuro.2003.10.012.

Farrell, M., Karp, B.I., Kassavetis, P., et al., 2020. Management of anterocapitis and anterocollis: a novel ultrasound guided approach combined with electromyography for botulinum toxin injection of Longus Colli and Longus Capitis. Toxins 12 (10). https://doi.org/10.3390/toxins12100626.

Geenen, C., Consky, E., Ashby, P., 1996. Localizing muscles for botulinum toxin treatment of focal hand dystonia. Can. J. Neurol. Sci. 23 (3), 194–197. https://doi.org/10.1017/s0317167100038506.

Gracies, J.M., Lugassy, M., Weisz, D.J., Vecchio, M., Flanagan, S., Simpson, D.M., 2009. Botulinum toxin dilution and endplate targeting in spasticity: a double-blind controlled study. Arch. Phys. Med. Rehabil. 90 (1), 9–16 e2. https://doi.org/10.1016/j.apmr.2008.04.030.

Grigoriu, A.I., Dinomais, M., Remy-Neris, O., Brochard, S., 2015. Impact of injection-guiding techniques on the effectiveness of botulinum toxin for the treatment of focal spasticity and dystonia: a systematic review. Arch. Phys. Med. Rehabil. 96 (11), 2067–20678 e1. https://doi.org/10.1016/j.apmr.2015.05.002.

Haig, A.J., Goodmurphy, C.W., Harris, A.R., Ruiz, A.P., Etemad, J., 2003. The accuracy of needle placement in lower-limb muscles: a blinded study. Arch. Phys. Med. Rehabil. 84 (6), 877–882. https://doi.org/10.1016/s0003-9993(03)00014-5.

Hallgren, R.C., Andary, M.T., Wyman, A.J., Rowan, J.J., 2008. A standardized protocol for needle placement in suboccipital muscles. Clin. Anat. 21 (6), 501–508. https://doi.org/10.1002/ca.20660.

Hauret, I., Dobija, L., Givron, P., Goldstein, A., Pereira, B., Coudeyre, E., 2023. Effectiveness of ultrasound-guided VS electrical-stimulation-guided botulinum toxin injections in triceps Surae spasticity after stroke: a randomized controlled study. J. Rehabil. Med. 55, jrm11963. https://doi.org/10.2340/jrm.v55.11963.

Heinen, F., Molenaers, G., Fairhurst, C., et al., 2006. European consensus table 2006 on botulinum toxin for children with cerebral palsy. Eur. J. Paediatr. Neurol. 10 (5–6), 215–225. https://doi.org/10.1016/j.ejpn.2006.08.006.

Henzel, M.K., Munin, M.C., Niyonkuru, C., Skidmore, E.R., Weber, D.J., Zafonte, R.D., 2010. Comparison of surface and ultrasound localization to identify forearm flexor muscles for botulinum toxin injections. Pharm. Manag. PM R 2 (7), 642–646. https://doi.org/10.1016/j.pmrj.2010.05.002.

Hong, J.S., Sathe, G.G., Niyonkuru, C., Munin, M.C., 2012. Elimination of dysphagia using ultrasound guidance for botulinum toxin injections in cervical dystonia. Muscle Nerve 46 (4), 535–539. https://doi.org/10.1002/mus.23409.

Im, S., Park, J.H., Son, S.K., Shin, J.E., Cho, S.H., Park, G.Y., 2014. Does botulinum toxin injection site determine outcome in post-stroke plantarflexion spasticity? Comparison study of two injection sites in the gastrocnemius muscle: a randomized double-blind controlled trial. Clin. Rehabil. 28 (6), 604–613. https://doi.org/10.1177/0269215513514983.

Jankovic, J., 2001. Needle EMG guidance for injection of botulinum toxin. Needle EMG guidance is rarely required. Muscle Nerve 24 (11), 1568–1570. https://doi.org/10.1002/mus.1186.

Jankovic, J., Orman, J., 1987. Botulinum A toxin for cranial-cervical dystonia: a double-blind, placebo-controlled study. Neurology 37 (4), 616–623. https://doi.org/10.1212/wnl.37.4.616.

Jost, W., 2019. Atlast of Botulinum Toxin Injection, third ed. Quintessence Pub Co.

Karp, B.I., Alter, K., 2017. Muscle selection for focal limb dystonia. Toxins 10 (1). https://doi.org/10.3390/toxins10010020.

Kaushik, P.S., Gowda, V.K., Shivappa, S.K., Mannapur, R., Jaysheel, A., 2018. A randomized control trial of botulinum toxin A administration under ultrasound guidance against manual palpation in spastic cerebral palsy. J. Pediatr. Neurosci. 13 (4), 443–447. https://doi.org/10.4103/JPN.JPN_60_18.

Kim, B.S., Kim, D.S., Kang, S., et al., 2021. Ultrasound-guided injection of the sternocleidomastoid muscle: a cadaveric study with implications for chemodenervation. Pharm. Manag. PM R 13 (5), 503–509. https://doi.org/10.1002/pmrj.12463.

Ko, Y.D., Yun, S.I., Ryoo, D., Chung, M.E., Park, J., 2020. Accuracy of ultrasound-guided and non-guided botulinum toxin injection into neck muscles involved in cervical dystonia: a cadaveric study. Ann Rehabil Med 44 (5), 370–377. https://doi.org/10.5535/arm.19211.

Kreisler, A., Simonin, C., Degardin, A., Mutez, E., Defebvre, L., 2020. Anatomy-guided injections of botulinum neurotoxin in neck muscles: how accurate is needle placement? Eur. J. Neurol. 27 (11), 2142–2146. https://doi.org/10.1111/ene.14415.

Kreisler, A., Gerrebout, C., Defebvre, L., Demondion, X., 2021. Accuracy of non-guided versus ultrasound-guided injections in cervical muscles: a cadaver study. J. Neurol. 268 (5), 1894–1902. https://doi.org/10.1007/s00415-020-10365-w.

Kreisler, A., Djelad, S., Simonin, C., et al., 2022. Does ultrasound-guidance improve the outcome of botulinum toxin injections in cervical dystonia? Rev. Neurol. (Paris) 178 (6), 591–602. https://doi.org/10.1016/j.neurol.2021.11.005.

Kutschenko, A., Klietz, M., Paracka, L., et al., 2020. Dysphagia in cervical dystonia patients receiving optimised botulinum toxin therapy: a single-center retrospective cohort study. J. Neural Transm. 127 (8), 1161–1165. https://doi.org/10.1007/s00702-020-02220-z.

Kwon, J.Y., Hwang, J.H., Kim, J.S., 2010. Botulinum toxin a injection into calf muscles for treatment of spastic equinus in cerebral palsy: a controlled trial comparing sonography and electric stimulation-guided injection techniques: a preliminary report. Am. J. Phys. Med. Rehabil. 89 (4), 279–286. https://doi.org/10.1097/PHM.0b013e3181ca24ac.

Lapatki, B.G., van Dijk, J.P., van de Warrenburg, B.P., Zwarts, M.J., 2011. Botulinum toxin has an increased effect when targeted toward the muscle's endplate zone: a high-density surface EMG guided study. Clin. Neurophysiol. 122 (8), 1611–1616. https://doi.org/10.1016/j.clinph.2010.11.018.

Lees, A.J., Turjanski, N., Rivest, J., Whurr, R., Lorch, M., Brookes, G., 1992. Treatment of cervical dystonia hand spasms and laryngeal dystonia with botulinum toxin. J. Neurol. 239 (1), 1–4. https://doi.org/10.1007/BF00839202.

Loens, S., Bruggemann, N., Steffen, A., Baumer, T., 2020. Localization of salivary glands for botulinum toxin treatment: ultrasound versus landmark guidance. Mov. Disord. Clin. Pract. 7 (2), 194–198. https://doi.org/10.1002/mdc3.12881.

Lungu, C., Nmashie, A., George, M.C., et al., 2022. Comparison of ultrasound and electrical stimulation guidance for onabotulinum Toxin-A injections: a randomized crossover study. Mov. Disord. Clin. Pract. 9 (8), 1055–1061. https://doi.org/10.1002/mdc3.13546.

Mayer, N.H., Whyte, J., Wannstedt, G., Ellis, C.A., 2008. Comparative impact of 2 botulinum toxin injection techniques for elbow flexor hypertonia. Arch. Phys. Med. Rehabil. 89 (5), 982–987. https://doi.org/10.1016/j.apmr.2007.10.022.

Molloy, F.M., Shill, H.A., Kaelin-Lang, A., Karp, B.I., 2002. Accuracy of muscle localization without EMG: implications for treatment of limb dystonia. Neurology 58 (5), 805–807. https://doi.org/10.1212/wnl.58.5.805.

Montminy, T., Belkind-Gerson, J., Kilgore, A., Tran, P., Mark, J.A., Children's Hospital Colorado, D.H.I., 2022. Evaluating the accuracy of anal botulinum toxin injection in children with constipation using endoscopic ultrasound. J. Pediatr. Gastroenterol. Nutr. 74 (4), e98. https://doi.org/10.1097/MPG.0000000000003397.

Picelli, A., Tamburin, S., Bonetti, P., et al., 2012a. Botulinum toxin type A injection into the gastrocnemius muscle for spastic equinus in adults with stroke: a randomized controlled trial comparing manual needle placement, electrical stimulation and ultrasonography-guided injection techniques. Am. J. Phys. Med. Rehabil. 91 (11), 957–964. https://doi.org/10.1097/PHM.0b013e318269d7f3.

Picelli, A., Bonetti, P., Fontana, C., et al., 2012b. Accuracy of botulinum toxin type A injection into the gastrocnemius muscle of adults with spastic equinus: manual needle placement and electrical stimulation guidance compared using ultrasonography. J. Rehabil. Med. 44 (5), 450–452. https://doi.org/10.2340/16501977-0970.

Picelli, A., Lobba, D., Midiri, A., et al., 2014. Botulinum toxin injection into the forearm muscles for wrist and fingers spastic overactivity in adults with chronic stroke: a randomized controlled trial comparing three injection techniques. Clin. Rehabil. 28 (3), 232–242. https://doi.org/10.1177/0269215513497735.

Pires, M., Saldanha, J., Claro, S., 2023. Ultrasound guidance superiority in pediatric sialorrhea treatment with intraglandular botulinum toxin application: a four-year retrospective study. Cureus 15 (9), e45359. https://doi.org/10.7759/cureus.45359.

Ploumis, A., Varvarousis, D., Konitsiotis, S., Beris, A., 2014. Effectiveness of botulinum toxin injection with and without needle electromyographic guidance for the treatment of spasticity in hemiplegic patients: a randomized controlled trial. Disabil. Rehabil. 36 (4), 313–318. https://doi.org/10.3109/09638288.2013.791727.

Py, A.G., Zein Addeen, G., Perrier, Y., Carlier, R.Y., Picard, A., 2009. Evaluation of the effectiveness of botulinum toxin injections in the lower limb muscles of children with cerebral palsy. Preliminary prospective study of the advantages of ultrasound guidance. Ann Phys Rehabil Med 52 (3), 215–223. https://doi.org/10.1016/j.rehab.2009.02.005.

Santamato, A., Micello, M.F., Panza, F., et al., 2014. Can botulinum toxin type A injection technique influence the clinical outcome of patients with post-stroke upper limb spasticity? A randomized controlled trial comparing manual needle placement and ultrasound-guided injection techniques. J. Neurol. Sci. 347 (1–2), 39–43. https://doi.org/10.1016/j.jns.2014.09.016.

Schiano, T.D., Fisher, R.S., Parkman, H.P., Cohen, S., Dabezies, M., Miller, L.S., 1996. Use of high-resolution endoscopic ultrasonography to assess esophageal wall damage after pneumatic dilation and botulinum toxin injection to treat achalasia. Gastrointest. Endosc. 44 (2), 151–157. https://doi.org/10.1016/s0016-5107(96)70132-3.

Schnitzler, A., Roche, N., Denormandie, P., Lautridou, C., Parratte, B., Genet, F., 2012. Manual needle placement: accuracy of botulinum toxin A injections. Muscle Nerve 46 (4), 531–534. https://doi.org/10.1002/mus.23410.

Scott, A.B., 1980. Botulinum toxin injection into extraocular muscles as an alternative to strabismus surgery. Ophthalmology 87 (10), 1044–1049. https://doi.org/10.1016/s0161-6420(80)35127-0.

Shaari, C.M., Sanders, I., 1993. Quantifying how location and dose of botulinum toxin injections affect muscle paralysis. Muscle Nerve 16 (9), 964–969. https://doi.org/10.1002/mus.880160913.

So, J.I., Song, D.H., Park, J.H., et al., 2017. Accuracy of ultrasound-guided and non-ultrasound-guided botulinum toxin injection into cadaver salivary glands. Ann Rehabil Med 41 (1), 51–57. https://doi.org/10.5535/arm.2017.41.1.51.

Speelman, J.D., Brans, J.W., 1995. Cervical dystonia and botulinum treatment: is electromyographic guidance necessary? Mov. Disord. 10 (6), 802. https://doi.org/10.1002/mds.870100619.

Stecco, C., Martinoli, C., Zampieri, D., et al., 2021. Subscapular botulinum injection for treatment of spasticity: ultrasound guide or not? A lesson from cadaver laboratory. Am. J. Phys. Med. Rehabil. 100 (2), e24–e25. https://doi.org/10.1097/PHM.0000000000001462.

Svetel, M., Vasic, M., Dragasevic, N., Pekmezovic, T., Petrovic, I., Kostic, V., 2009. Botulinum toxin in the treatment of sialorrhea. Vojnosanit. Pregl. 66 (1), 9–12. https://doi.org/10.2298/vsp0901009s.

Turna, I.F., Erhan, B., Gunduz, N.B., Turna, O., 2020. The effects of different injection techniques of botulinum toxin a in post-stroke patients with plantar flexor spasticity. Acta Neurol. Belg. 120 (3), 639–643. https://doi.org/10.1007/s13760-018-0969-x.

Tyslerowicz, M., Jost, W.H., 2019. Injection into the Longus Colli muscle via the thyroid gland. Tremor Other Hyperkinet Mov (N Y) 9. https://doi.org/10.7916/tohm.v0.718.

Tyslerowicz, M., Dulski, J., Gawryluk, J., Slawek, J., 2022. Does ultrasound guidance improve the effectiveness of neurotoxin injections in patients with cervical dystonia? (A prospective, partially-blinded, clinical study). Toxins 14 (10). https://doi.org/10.3390/toxins14100674.

Unal, S., Tugra Karaarslan-Turk, F., Akbostanci, M.C., Peker, E., Yilmaz, R., 2022. Botulinum toxin injections in jaw-opening dystonia. The lateral pterygoid - maxillary artery problem. J. Clin. Neurosci. 101, 217–220. https://doi.org/10.1016/j.jocn.2022.05.017.

Van Campenhout, A., Molenaers, G., 2011. Localization of the motor endplate zone in human skeletal muscles of the lower limb: anatomical guidelines for injection with botulinum toxin. Dev. Med. Child Neurol. 53 (2), 108–119. https://doi.org/10.1111/j.1469-8749.2010.03816.x.

Vejbrink Kildal, V., Rodriguez-Lorenzo, A., Pruidze, P., et al., 2023. Ultrasound-guided injections for treatment of facial paralysis sequelae: a randomized study on body donors. Plast. Reconstr. Surg. https://doi.org/10.1097/PRS.0000000000010802.

Wissel, J., Ward, A.B., Erztgaard, P., et al., 2009. European consensus table on the use of botulinum toxin type A in adult spasticity. J. Rehabil. Med. 41 (1), 13–25. https://doi.org/10.2340/16501977-0303.

Wu, C., Xue, F., Chang, W., et al., 2016. Botulinum toxin type A with or without needle electromyographic guidance in patients with cervical dystonia. SpringerPlus 5 (1), 1292. https://doi.org/10.1186/s40064-016-2967-x.

Xu, K., Yan, T., Mai, J., 2009. A randomized controlled trial to compare two botulinum toxin injection techniques on the functional improvement of the leg of the children with cerebral palsy. Clin. Rehabil. 23, 800–811.

Yang, E.J., Rha, D.W., Yoo, J.K., Park, E.S., 2009. Accuracy of manual needle placement for gastrocnemius muscle in children with cerebral palsy checked against ultrasonography. Arch. Phys. Med. Rehabil. 90 (5), 741–744. https://doi.org/10.1016/j.apmr.2008.10.025.

Yun, J.S., Chung, M.J., Kim, H.R., et al., 2015. Accuracy of needle placement in cadavers: non-guided versus ultrasound-guided. Ann Rehabil Med 39 (2), 163–169. https://doi.org/10.5535/arm.2015.39.2.163.

Zeuner, K.E., Knutzen, A., Kuhl, C., et al., 2017. Functional impact of different muscle localization techniques for botulinum neurotoxin A injections in clinical routine management of post-stroke spasticity. Brain Inj. 31 (1), 75–82. https://doi.org/10.1080/02699052.2016.1218545.